Brief report

Use of B cell–bound HLA-A2 class I monomers to generate high-avidity, allo-restricted CTLs against the leukemia-associated protein Wilms tumor antigen


Recent studies have detected Wilms tumor antigen (WT1)–specific cytotoxic T lymphocytes (CTLs) in patients with acute myelogenous leukemia (AML) and chronic myelogenous leukemia (CML) and demonstrated that most of these CTLs were low avidity. Although HLA-mismatched donors can mount high-avidity CTLs against HLA-A2–presented peptides of WT1, a dominant alloimmune response usually obscures detection of peptide-specific CTLs. Here we explored the feasibility of using recombinant HLA-A2 monomers containing single peptide epitopes as immunogens to generate peptide-specific CTLs from allogeneic donors. We demonstrate that the coating of HLA-A2–B lymphocytes with A2/peptide monomers provides a strong stimulus for autologous peptide-specific CTLs. After 3 to 5 rounds of stimulation a population of CD8+ T cells binding A2/peptide tetramers is easily detectable by fluorescence-activated cell sorting analysis. Furthermore, sorted A2/WT1 tetramer-positive CTLs display strong cytotoxic activity against leukemia cells expressing WT1 endogenously but not against WT1+ human tumor cells. Thus, HLA/peptide monomers may be useful to isolate peptide-specific donor lymphocytes for treatment of patients with leukemia after HLA-mismatched transplantation. (Blood. 2004;103:4613-4615)

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Introduction

Wilms tumor antigen (WT1) is expressed at high levels in patients with chronic myelogenous leukemia (CML), acute myelogenous leukemia (AML), and acute lymphocytic leukemia (ALL) and can serve as a marker for disease burden. Because WT1 is expressed at low levels in some normal tissues including CD34+ hematopoietic progenitor/stem cells, it is likely that tolerance mechanisms may render high-avidity cytotoxic T lymphocytes (CTLs) unresponsive. Although 2 recent reports provided evidence for WT1-specific CTLs in patients with leukemia, in one of these studies it was demonstrated that low-avidity CTLs were expanded in patients with CML. In the past, we have shown that the T-cell repertoire of HLA-mismatched donors can be used to isolate high-avidity CTLs against HLA-A2–presented peptide epitopes of WT1. However, in our experience the isolation of such peptide-specific CTLs is unsuccessful in many donors because allogeneic stimulator cells often provoke dominant CTL responses against allogeneic epitopes unrelated to the A2-presented WT1 epitope.

In this report we have explored the possibility of avoiding allogeneic stimulator cells and use instead recombinant HLA-A2/peptide monomers as immunogens. Single-chain streptavidin-labeled antibodies that specifically bind to CD20 molecules were used to provide a binding site for biotinylated HLA monomers on the surface of B lymphocytes. This allowed us to introduce allogeneic HLA-A2/peptide complexes as the sole antigen into peripheral blood mononuclear cell (PBMC) cultures of HLA-A2+ donors and to exploit autologous B lymphocytes as antigen-presenting cells (APCs).

Study design

Antibodies

The recombinant scFvSA fusion protein has been previously described.12 This protein, referred to here as anti-CD20SA, consists of the heavy- and light-chain variable regions of the anti-CD20 antibody B9E9 joint to the streptavidin sequence of Streptomyces avidinii.

Cells

PBMCs from 4 HLA-A2+ healthy donors were used after obtaining informed consent. The study was approved by the Velindre Hospital institutional review board. T2 is a transport-associated protein (TAP)–deficient human HLA-A2+ cell line.13 BV173 and ZR571 are HLA-A2+ human leukemia and breast cancer lines. CIR and C1R-A2 are HLA-A2+ and A2+ Epstein-Barr virus (EBV)–transformed human cell lines. All cells were maintained in RPMI plus 10% fetal

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calf serum (FCS). In this report HLA-A2⁺ cells and monomers/tetramers are of A*0201 subtype.

Peptides

The following HLA-A2-binding peptides were used: the WT1-derived peptide RMFPNAPYL (referred to as pWT126),³⁰ the Melan-A-derived peptide ELA-GIGILTV (pMelA),¹⁴ influenza virus matrix peptide GILGFVFTL (pFlu),³¹ the HIV-Gag peptide SLYNTVAL (pHIV),³⁰ the telomerase peptide ILAKFHWLYL (pTel),¹⁷ and the HPV16 E7 peptide YMLDLQPETT (pHPV).¹⁴ In addition the HLA-A1–binding tyrosinase peptide KSDICTDEY (pTyR)⁹⁹ and HLA-A3–binding bcr-abl peptide ATGFKQSSK (pBcr)²⁰ were used.

HLA-A2/peptide complex monomers and tetramers

Recombinant biotinylated HLA-A2 class I monomers and A2 or A3 or B7 fluorescent tetramers containing the peptides as described in the previous section were obtained from ProImmune (Oxford, United Kingdom).

In vitro immunization protocol

PBMCs were incubated with anti-CD20SA (10 µg/mL) diluted in phosphate-buffered saline (PBS) for 1 hour at room temperature, washed, and incubated with the biotinylated A2/peptide monomers (0.5 µg/mL in PBS) for 30 minutes at room temperature, washed, and plated at 3 x 10⁶ cells/well in 24-well plates in RPMI with 10% human AB serum. Interleukin 7 (IL-7; R&D Systems, Minneapolis, MN) was added on day 1 at 10 ng/mL and IL-2 (Chiron, Harefield, United Kingdom) was added at 10 U/mL on day 4.¹⁵ Over a 5-week period cells were restimulated weekly with fresh PBMCs, mixed with responder cells at a 1:1 ratio, and plated at 3 x 10⁶/well in 24-well plates.

FACS staining/sorting

Approximately 10⁵ PBMCs were incubated with tetramer-phycocerythrin (PE) for 30 minutes at 37°C, followed by anti-CD8-fluorescein isothiocyanate (FITC)/allophycocyanin (APC) for 20 minutes at 4°C, followed by fluorescence-activated cell sorting (FACS)–Calibur analysis. Sorting was done with a FACS-Vantage (Becton Dickinson, Cowley, Oxford, United Kingdom). Sorted tetramer-positive cells were expanded in 24-well plates using, per well, 2 x 10⁵ sorted cells, 2 x 10⁴ irradiated A2⁺ PBMCs as feeders, 2 x 10⁹ CD3/CD28 beads/mL (Dynal, Oslo Norway), and IL-2 (1000 U/mL).

Chromium-release assay

CTL assays were performed as described previously and the specific lysis was calculated as: % lysis = experimental cpm – spontaneous cpm/maximum cpm – spontaneous cpm x 100.

Results and discussion

It has been shown previously that recombinant HLA-A2 monomer/peptide complexes can be used to stimulate peptide-specific, self-HLA-restricted CTLs.³³ In this study we tested if monomers can generate HLA-A2-restricted CTLs specific for leukemia-associated peptides in HLA-A2⁺ donors. This is relevant for the development of peptide-specific immunotherapy options for patients with leukemia undergoing HLA-mismatched stem cell transplantation.

We focused on 2 HLA-A2–presented CTL epitopes, a WT1-derived peptide pWT126⁰ and a Melan-A-derived peptide, pMelA.¹⁴ WT1 is a target for immunotherapy of leukemia, whereas Melan-A can function as a target in melanoma. In the self-restricted setting CTLs against WT1 are rare,⁹ whereas CTLs against Melan-A are frequent.²³ Using anti-CD20SA antibodies B lymphocytes of HLA-A2⁺ donors were decorated with A2/peptide monomers and used as APCs for autologous T lymphocytes. After 4 rounds of stimulation with pMelA monomers, FACS analysis revealed that nearly 16% of CD8⁺ cells stained with pMelA tetramers but not with a panel of control tetramers (Figure 1A-E), and cytotoxicity assays revealed that the CTLs displayed pMelA-specific killing activity (Figure 1F). Similarly, FACS analysis of cultures stimulated 5 times with pWT126 monomers showed up to 9.5% of CD8⁺ cells staining specifically with A2/pWT126 tetramers (Figure 1G-K) and cytotoxicity assays revealed pWT126-specific killing activity (Figure 1L). In total 3 HLA-A2⁻ donors were stimulated with pWT126 monomers resulting in the generation of pWT126-specific CTLs, as determined by tetramer staining, in all donors after 4 or 5 stimulations (range, 0.7%–9.5% tetramer-positive CD8⁺ cells). Four donors were stimulated with pMelA monomers and all developed specific CTLs after 3 to 5 stimulations (range, 5.5%–23% tetramer-positive CD8⁺ cells). In addition 2 donors were stimulated with pFlu monomers and one donor with pHPV monomers resulting in the generation of CTLs specific for these epitopes in all cases (data not shown). Although this indicates that monomers can reliably prime and expand peptide-specific CTLs, a side-by-side comparison would be required to compare the efficiency of CTL induction by T2 cells and monomers.

Stimulation with allogeneic monomers may also expand high-avidity CTLs that bind the allogeneic monomer independent of
presented peptide. We found that such high-avidity, peptide-independent, alloreactive CTLs have a distinct tetramer staining profile. For example, Figure 2A–H shows the analysis of a bulk culture stimulated with A2/pWT126 monomers. In this culture CD8+ cells bind a panel of A2 tetramers in a peptide nonspecific fashion, but not HLA-A1 and A3 tetramers. The individual A2 tetramers appeared to bind to the same CD8+ population because the percentage of positive cells did not increase when all tetramers were combined (Figure 2F). Although we cannot rule out tetramer binding to the inhibitory receptor immunoglobulin-like transcript 2 (ILT2), the lack of binding of HLA-A1/A3 tetramers suggests that this is unlikely.21 Together, the data suggest that tetramer analysis can serve to identify cultures containing high-avidity pan-A2-alloreactive CTLs, which would be expected to pose a high risk of graft-versus-host disease if used for donor lymphocyte infusion in patients after transplantation. In this study we found that allogeneic monomers stimulated more often peptide-specific CTLs (10 cases) than pan-A2-alloreactive CTLs (1 case).

Finally, we examined whether CTLs that specifically bind A2/pWT126 tetramers were of sufficient avidity to kill human leukemia cells expressing WT1 endogenously. Cell sorting was used to enrich for tetramers were of sufficient avidity to kill human leukemia cells expressing WT1 endogenously. Cell sorting was used to enrich for pan-A2 alloreactive CTLs. Although we cannot rule out tetramer binding to the inhibitory receptor immunoglobulin-like transcript 2 (ILT2), the lack of binding of HLA-A1/A3 tetramers suggest that this is unlikely. Together, the data suggest that tetramer analysis can serve to identify cultures containing high-avidity pan-A2-alloreactive CTLs, which would be expected to pose a high risk of graft-versus-host disease if used for donor lymphocyte infusion in patients after transplantation. In this study we found that allogeneic monomers stimulated more often peptide-specific CTLs (10 cases) than pan-A2-alloreactive CTLs (1 case).

In summary, we have presented data showing that allogeneic monomorphic HLA class I peptide complexes presented by autologous B lymphocytes can stimulate and expand peptide-specific alloreactive CTLs. If produced to Good Manufacturing Practices (GMP) grade, HLA/peptide monomers may become useful reagents to enrich for peptide-specific donor lymphocytes for the treatment of patients with leukemia following HLA-mismatched transplantation.

References

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